Sequential Analysis of 43 Patients With Non-Hodgkin's Lymphoma: Clinical Correlations With Cytogenetic, Histologic, Immunophenotyping, and Molecular Studies

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Few reports correlating specific cytogenetic abnormalities with distinct subtypes of lymphoma have performed serial studies at diagnosis and at tumor recurrence or progression. In our file of 325 cytogenetically analyzed non-Hodgkin's lymphoma (NHL) patients studied over the past decade, 43 had serial biopsies, 39 of whom had at least two successful preparations; of the 43, nine had one and 32 had two or more cytogenetically abnormal specimens. In this study, we correlated cytogenetic, histopathologic, molecular, and clinical parameters. Patients with low-grade lymphomas were as likely as patients with intermediate- or high-grade lymphomas to acquire new chromosomal abnormalities with time (16 of 23 patients as compared with 7 of 16; P_2 = .11, χ^2 test). In four patients, originally diagnosed indolent disease progressed to aggressive disease; all had t(14;18), all gained additional chromosomal abnormalities with disease progression, and three of the four expressed abnormalities associated with disease progression and/or short survival: der(18), +7, and/or +12. Cytogenetic results from early disease were compared with those obtained later in disease: in the t(14;18) group, the most common abnormalities were +7 (eight patients) and der(18) (five patients), both seen later in disease. The most common abnormalities in patients without t(14;18) were 6q deletions; they were seen in both early and late disease and were associated with significantly shorter survivals (P_2 = .0014) compared with all patients without 6q deletions. Secondary chromosomal abnormalities, observed after at least one previous abnormal study, were seen in 19 of 22 t(14;18) patients and in 11 of 21 patients without t(14;18) and were associated with a poor survival (P_2 = .13) compared with patients without any secondary chromosomal abnormalities. Chromosome 1 abnormalities were seen in almost half of the patients and were observed in initial specimens and early in disease as well as late in disease and as secondary abnormalities; 1q involvement was more frequent than 1p (15 versus eight patients) and was significantly associated with poor survival only in patients with intermediate-/high-grade disease; the most common breakpoints were 1q21-q22 (nine patients) and 1p36 (six patients). Breakpoints at 2q21 and 3q27-q29 were limited to patients with t(14;18) and were almost exclusively secondary in nature. Molecular studies in 24 of our patients showed discrepancies with the cytogenetic results in only three patients: two had t(14;18) but no molecular rearrangements while two patients had no visible t(14;18) but were positive for major breakpoint region (MBR) rearrangement. The presence of MBR or minor breakpoint cluster (MCR) rearrangement had no apparent effect on survival. The most significant prognostic indicator was low-grade lymphoma ($P_2 < .0001$). Age (P_2 = .012) and the presence of 1q (P_2 = .0026) in intermediate-/high-grade disease, and the presence of t(14;18) in low-grade lymphoma (P_2 = .035) were prominent prognostic variables in the two grade categories.

SEVERAL LARGE series of cytogenetic studies in lymphoma have been reported.[1-11] In contrast to Hodgkin's disease,[1] the non-Hodgkin's lymphomas (NHLs) demonstrate considerable histologic, immunologic, and cytogenetic heterogeneity. A number of consistent cytogenetic abnormalities have been associated with particular subtypes of lymphomas, such as the 14;18 translocation in follicular lymphomas, the 11;14 translocation in mantle-cell lymphomas, 3q27 abnormalities in large-cell lymphomas, and 8q24 abnormalities in Burkitt's lymphomas. Not only do these characteristic abnormalities serve as markers for specific types of lymphomas, but their identification has also led to the discovery of important oncogenes (eg, *myc*, *bcl*-2, *bcl*-1, and *bcl*-6) that are now implicated in the pathogenesis of these lymphomas and other tumors.

Although it is well known that neoplasms acquire secondary cytogenetic abnormalities as they recur and/or progress, only a few investigators[3,4,6] have reported serial studies with tumor recurrence or progression in lymphoma. The investigation of secondary changes is of interest with regard to the potential prognostic implications of these changes; they may also yield important information regarding genetic loci involved in tumor progression. As there is little information regarding the frequency and specificity of secondary changes and their relationship to clinical course, we examined a series of patients with multiple studies. In our file of 325 cytogenetically analyzed lymphoma patients studied over the past decade, 43 had sequential biopsies. In this report, we compare the results of cytogenetic, histologic, and molecular studies in relation to clinical course, prognosis, and survival.

MATERIALS AND METHODS

Serial studies of lymph nodes or other involved sites were performed on a total of 43 patients with NHL who were treated and observed in the Medicine Branch, Clinical Center, National Institutes of Health (Bethesda, MD). Of these 43 patients, 25 were classified as low-grade, 10 as intermediate-grade, and eight as high-grade

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Table 1. Cytogenetic Specimens in Serial Studies

Specimen Type	No. Patients	No. Specimens	No. Successful	No. Abnormal*
Lymph node	43	99	86	69
Bone marrow	25	34	28	4†
Peripheral blood	8	10	8	3‡
Spleen	7	7	3	3
Pleural effusion	4	8	4	3
Ascites fluid	1	2	2	2
Cerebrospinal fluid	1	2	0	0

*Cytogenetically abnormal.

† One patient had Burkitt's lymphoma, two had bone marrow involvement with lymphoma, and one had a secondary hematologic disorder.

‡ One patient had Burkitt's lymphoma, one patient had lymphoma involvement, and one patient had T-cell lymphoblastic lymphoma.

lymphoma. Biopsy materials were divided for histopathology, cytogenetic analysis, cell-surface marker analysis, and molecular gene rearrangement studies. All patients were histologically classified according to the International Working Formulation.[12] Indolent lymphomas include all low-grade categories of the Working Formulation plus mantle-cell lymphomas, most equivalent to diffuse, small cleaved cell type; aggressive lymphomas include intermediate- and high-grade lymphomas. All lymphomas were immunophenotyped according to previously published techniques. Early studies include those done at diagnosis or within 1 month of diagnosis (case 32, first studied at 2 months postdiagnosis, did not receive chemotherapy before study).

A total of 99 lymph nodes were analyzed. In addition, samples of bone marrow (total 34), peripheral blood, spleen, pleural effusion, ascites fluid, and cerebrospinal fluid were also submitted for analysis. The number of specimens for each type, as well as the numbers of successful and abnormal specimens, are listed in Table 1.

Cytogenetic studies. Single cell suspensions of the lymph nodes were obtained by a variety of techniques including mincing with scissors, followed by passage through stainless steel mesh, use of a homogenizer, or repeated aspiration through a 22-gauge needle. Use of collagenase or other means of disaggregation are not deemed necessary for this tissue. The lymph node cells were either processed directly without culture or cultured in vitro for 1 to 5 days with and without mitotic stimulation. Mitogens used included phytohemagglutinin (PHA), pokeweed mitogen (PWM), interleukin-2 (IL-2), phorbol-12-myristate-12 acetate (TPA), and lipopolysaccharde (LPS), which were added either singly or in combination. Epstein-Barr virus (EBV) was used in a few selected B-cell malignancies. In general, the most successful samples were those cultured for 1 to 2 days without mitogenic stimulation. Bone marrow samples were processed directly and cultured in vitro for 1 day. The remaining tissues were processed directly and/or cultured for 1 to 4 days. All specimens were harvested according to standard techniques. One air-dried slide was stained with the standard Giemsa stain and was scored for modal chromosome number and frequency of chromosomal aberrations (breaks, fragments, rings, and minutes) that can be difficult to detect in banded preparations. The remaining slides were stained with Trypsin-Giemsa stain. A total of 20 to 30 metaphase cells were analyzed microscopically, and a minimum of five cells were karyotyped for each sample when possible. Karyotypes were prepared according to the International System for Human Cytogenetic Nomenclature (ISCN)-Guidelines for Cancer Cytogenetics.[13]

Molecular studies. The involvement of *bcl*-2 and *myc* was assessed by Southern blot analysis as previously described.[14] Involvement of *bcl*-2 was assessed using probes to the major breakpoint region (MBR), the minor breakpoint cluster (MCR), and a second minor breakpoint located 5′ to exon 2 that has been reported to occur rarely in follicular lymphomas and in about 5% of cases of chronic lymphocytic leukemia (CLL). Involvement of *myc* was assessed using a third exon probe.

Statistical methods. The probability of survival was calculated using the Kaplan-Meier method,[15] and the significance of the difference between pairs of Kaplan-Meier curves was calculated using the Mantel-Haenszel procedure.[16] The Cox proportional hazards model was used to identify which factors are jointly significant in their association with survival.[17] The resulting model parameters (b_i) were converted to relative risks by computing exp(b_i), where exp(a) = 2.7183^a.[18] The 95% confidence interval for the relative risk was computed as [exp(b_{iL}), exp(b_{iH})], where $b_{iL} = b_i - 1.96$ [estimated standard error (b_i)] and $b_{iH} = b_i + 1.96$ [estimated standard error (b_i)]. The relative risk indicates the risk associated with dying while being in a greater risk category compared with that of being in a lower risk category. All P values are two-sided and denoted by P_2.

RESULTS

All 43 patients had at least two successful studies (mean, 2.9 successful studies per patient; range, two to six. Of these 43 patients, nine patients had one study and 32 patients had two or more studies showing cytogenetic abnormalities. The numbers of successful and abnormal specimens for each type of tissue studied are presented in Table 1. Of the 99 submitted lymph nodes, 86 (87%) were successful, and 69 (80%) of these were cytogenetically abnormal. Approximately half of the spleens and effusions (various types) studied were successful, and 8/9 of these showed clonal abnormalities. Cytogenetic abnormalities were also seen in bone marrow and peripheral blood in a few cases (see notes to Table 1 for diagnoses); a majority of the bone marrows studied (22/34) were obtained at diagnosis or shortly thereafter and were both morphologically and cytogenetically normal. The time interval between specimen sampling in these patients varied considerably: the time between the first and last sample ranged from 4 to 159 months.

The 24 male patients and 19 female patients ranged in age (at diagnosis) from 10 to 65 years (median, 40 years). The median survival was 84.6 months (range, 5 to 197+ months). Using the Working Formulation, the 43 patients were divided into lymphoma subtypes A through J according to initial diagnosis and further subdivided into four cytogenetic groups according to the presence of certain clonal abnormalities: t(14;18), t(8;14), other abnormalities, and normal (Table 2). All but two patients (one each in groups B and C) in the t(14;18) group and all three patients in the t(8;14) group exhibited other clonal chromosomal abnormalities in addition to the specific translocation. The majority of patients exhibited pseudodiploidy or hyperdiploidy, most having 47 to 50 chromosomes; only three patients had hypodiploid clones, and three had tetraploid clones. Complete cytogenetic results are presented in the Appendix (in the same order as in Table 2), along with histologic diagnosis for each specimen.

Patients with the t(14;18) translocation had primarily low-grade lymphoma (18 of 22 patients), while all three patients

Table 2. Serial Studies: Summary of Cytogenetic Results According to National Cancer Institute Working Formulation of Non-Hodgkin's Lymphomas for Clinical Usage

Working Formulation	t(14;18)*	t(8;14)†	Other Abnormalities	Normal
Low grade				
A. Small lymphocytic			3 (72)	1 (122+)
B. Follicular, predominantly small cleaved cell	8 (110)		1 (61+)	
C. Follicular, mixed small cleaved and large-cell	10‡ (110)		1 (20)	1 (86+)
Intermediate grade				
D. Follicular, predominantly large-cell	1 (69+)			
E. Diffuse, small cleaved cell				
F. Diffuse, mixed small cleaved and large-cell			1 (18)	
G. Diffuse, large-cell	2§ (48)			
Malignant lymphoma, NOS‖		1 (9)	4 (31)	
		1¶ (31)		
High grade				
H. Large-cell, immunoblastic			2 (22)	
I. Lymphoblastic			3 (50)	
J. Small noncleaved cell	1 (12)	1 (5)	1# (100+)	
Total no. patients	22	3	16	2

Numbers in parentheses are median survivals.

* All patients with 14;18 had additional clonal chromosomal abnormalities, except one patient each in groups B and C.

† All patients with 8;14 had additional clonal chromosomal abnormalities.

‡ One patient had a complex 14;18 translocation: t(3;14)(14;18)(q29;q13q32;q21).

§ One patient had a complex 14;18 translocation: t(14;18;1)(q32;q21;q31).

‖ Wiscott-Aldrich syndrome → malignant lymphoma with plasmacytoid features, not further subclassified.

¶ This patient had a complex 8;14 translocation: t(6;8;14)(p21;q24;q32).

This patient later had lymph node diagnosed as lymphoid hyperplasia (survival, 100+ months).

with t(8;14) had more aggressive disease. Three patients had complex t(14;18) or t(8;14) translocations: case 33 had t(3;14)(14;18)(q29;q13q32;q21) (Fig 1), low-grade follicular lymphoma (group C) and a survival of 142+ months; case 15 had t(14;18;1)(q32;q21;q31), large-cell immunoblastic lymphoma (groups H → D) and a survival of 31 months; and case 28 had t(6;8;14)(p21;q24;q32), Wiscott-Aldrich syndrome and an unclassifiable lymphoma with plasmacytoid features, histologically intermediate grade, and a survival of 31 months. Case 30, with large-cell anaplastic Ki-1 lymphoma, had multiple abnormalities of chromosome 2, including der(5)t(2;5) (p23;q35), an abnormality reported in several cases of Ki-1 lymphoma.[19,20] Case 32, with angioimmunoblastic lymphadenopathy (AILD)-like T-cell lymphoma had del(8)(p21).

Of the five patients (cases 4, 24, 33, 36, and 41) with long survivals (142+ to 197+ months), all had t(14;18) (one was a complex translocation); two cases had t(1;12)(q21;q24) (Fig 1); and +7, −20, +21 were each seen in two patients. All five patients were originally diagnosed with indolent low-grade lymphoma. Five patients (cases 7, 10, 30, 35, and 40) had short survivals (median survival, 10 months; range, 5 to 12 months). All had multiple structural abnormalities, and although no specific common breakpoints were noted in these patients, all five had breakpoints in chromosome 1 (the breakpoint was different in each case, involving 1p in two patients and 1q in three patients). Two of the five patients had t(8;14), two had involvement of 13q13-14, one had t(14;18), and one had a homogeneously staining region on chromosome 11q (Fig 2).

In four patients, originally diagnosed indolent disease progressed to aggressive disease. All four had t(14;18), and all gained additional chromosomal abnormalities with disease progression. Other than the t(14;18), no abnormality was common to all four patients: two patients had secondary der(18), and one of these patients also had a 6q deletion, +7, and +12. A third patient had +7, +12, and +18.

Summarized in Table 3 are the most common abnormalities seen in patients with and without t(14;18) in early studies and studies performed later in the disease. The most common were those observed in t(14;18) patients later in their disease: +7 (eight patients), der(18) (five patients), and breakpoints at 1p36 and 6q13 (four patients each). Chromosomal instability did not appear to be greater in any particular subgroup of patients.

The frequency of secondary chromosomal abnormalities (ie, changes observed after at least one previous abnormal study) is shown in Fig 3: 19 of 22 patients with t(14;18) and 11 of 21 patients without t(14;18) demonstrated such changes ($P_2 = .015$ χ^2 test). The only breakpoints involved in structural changes that were observed in more than one patient were 1p36 (four patients), 2q21 (three patients), and 6q13-14 (three patients) in patients with t(14;18), and 3p26 and 3q24 (two patients each) in patients without t(14;18). The most frequent numerical secondary changes involved chromosomes 7, 20, 21, and 22 in patients with t(14;18). Five patients showed duplication of the 18q chromosome, termed der(18) in this report, derived from t(14;18) (q32;q21): in four of five patients it was seen later in disease, and in two of five it was seen as a secondary change. It was not associated with a shortened survival (median survival,

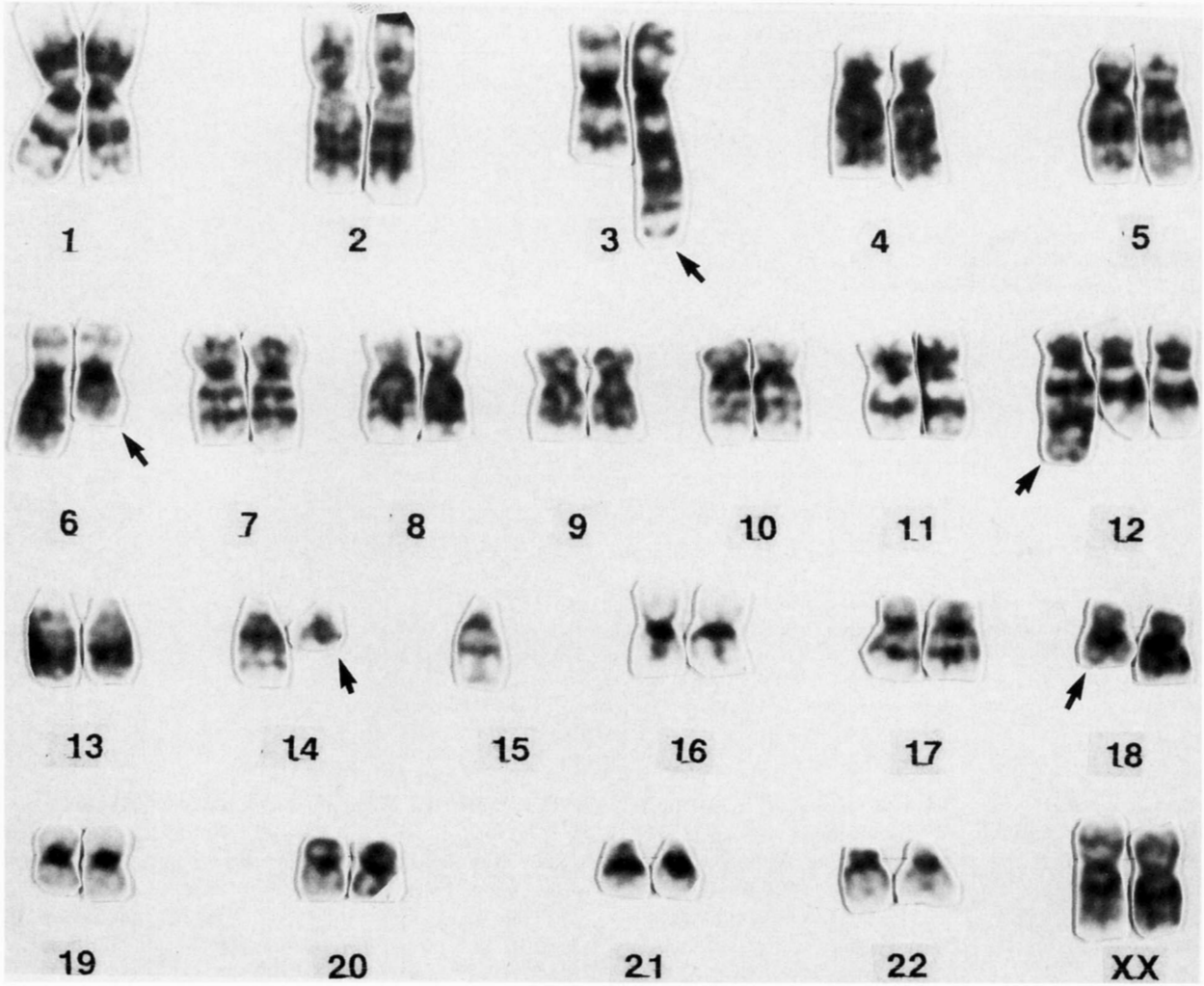

Fig 1. Karyotype from case 33 from a lymph node diagnosed as malignant lymphoma, follicular, mixed small-cell and large-cell (95 months after original diagnosis): 46,XX,t(3;14)(14;18)(q29;q13q32;q21),del(6)(q21),+der(12)t(1;12)(q21;q24),−15.

84 months; median survival after first observation of the 18q, 30 months). The only other recurring secondary structural abnormalities were del(6)(q13) (three patients) and i(6p) (two patients).

Figure 4 shows the overall breakpoint distribution in the 43 cases in this series (leukemic breakpoints in case 13 are not included). The most common breakpoints are, of course, 14q32 and 18q21 as a result of the 14;18 translocation, followed by breaks at 1q21 (eight patients), 1p36 (six patients), and 6q21 (five patients). Of a total of 13 patients with breaks in 6q, from 6q13 to 6q27, 10 exhibited 6q deletions (cases 1, 5, 10, 11, 15, 18, 19, 26, 33, and 38).

Immunophenotyping studies. All but five patients had B-cell lymphoma. The exceptions were three cases of precursor T-cell lymphoblastic lymphoma (cases 17, 23, and 38), one case of AILD-like T-cell lymphoma (case 32), and one case of Ki-1+, T-cell, large-cell anaplastic lymphoma (case 30).

Molecular breakpoint studies. A total of 24 patients were studied for *bcl*-2 rearrangement (Table 4). Thirteen patients had breaks in the MBR, and five patients were positive for breaks in the MCR. One patient (case 29) with no t(14;18) showed no detectable breakpoint in any of the known regions. Cases 22 and 26 were positive for MBR rearrangement yet showed no detectable chromosome 14;18 rearrangement. On the other hand, cases 2, 35, and 43 all exhibited t(14;18) but were negative for molecular evidence of breaks in MBR and MCR. These three cases were further analyzed for *bcl*-2 breakpoints in the rare 5′ breakpoint cluster and were negative.

Survival analysis results. The results of survival analysis of a number of parameters, including lymphoma stage and the most common recurring chromosomal abnormalities, are shown in Table 5 and Fig 5 through 9. The most significant factor was disease grade: low- versus intermediate-/high-grade ($P_2 < .0001$; Fig 5). When intermediate- and high-grade were compared, no significant difference was found ($P_2 = .76$), so they were combined. When the significance of t(14;18) alone, regardless of grade, was examined, its presence was a significant positive survival factor ($P_2 =$

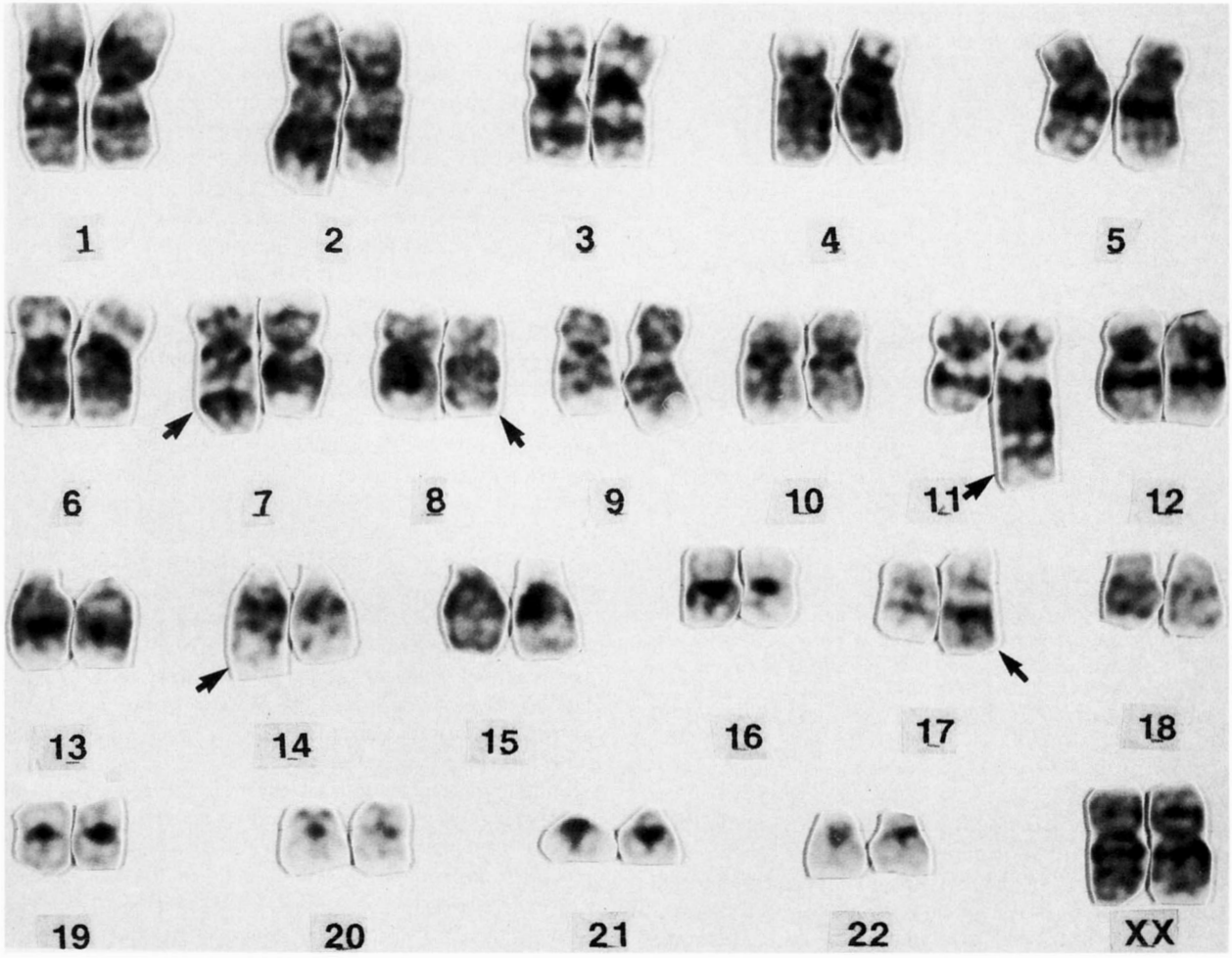

Fig 2. Karyotype from the peripheral blood of case 40 with Burkitt's lymphoma (5 months after diagnosis): 46,XX,der(7)t(2;7)(q32;q32), t(8;14)(q24;q32),der(11)t(11;HSR;2)(q23;HSR;q33),der(17)t(2;17)(q32;q21).

.0003); however, t(14;18) was found to be significant only in low-grade disease where patients with the t(14;18) had a better survival than those without this translocation (P_2 = .021; Fig 6). A number of chromosome 1 parameters were studied, including involvement of the whole chromosome, 1p, 1q, 1p36, and each type of aberration as a primary or secondary abnormality, and by disease grade. The most significant parameter was 1q involvement in intermediate-/high-grade disease: patients with 1q abnormalities had a shorter survival than those without 1q abnormalities, P_2 = .0078 (Fig 7). Studies of 6q abnormalities, including translocations, deletions, and specific breakpoints, revealed the most significant to be 6q deletions: patients with a 6q deletion but no t(14;18) (all were intermediate-/high-grade) had a significantly shorter survival than patients without this deletion (P_2 = .0014), or those with the deletion and t(14;18) (P_2 = .013; all were low-grade). One of the primary reasons for conducting serial studies was to determine the prognostic importance of secondary chromosomal abnormalities. As can be seen in Fig 9, the development of such abnormalities was a negative prognostic indicator (P_2 = .13). Trisomies of chromosomes 7 and 12 and tumor progression were not noted to be significant prognostic factors in this group of 43 patients.

Because of obvious prognostic differences between low-grade and intermediate-/high-grade NHL, separate Cox models were constructed for these two groups of patients (Table 6). For the intermediate-/high-grade patients, both age ($\leq$29 years) and the presence of chromosome 1q abnormalities were significant negative prognostic factors (P_2 = .012 and .0026, respectively). The Cox model for the low-grade patients indicated that only lack of t(14;18) was a significant negative prognostic factor. A model including this translocation plus age did show some effect of age (less than 29 years) as being a negative prognostic factor (P_2 = .081), along with a lack of t(14;18) (P_2 = .011).

DISCUSSION

The goal of this study was to examine sequential alterations in the cytogenetic profile of NHL over time. Our

Table 3. Most Common Chromosomal Abnormalities: Early Versus Later Studies

Patients with t(14;18) (22 patients)
Early study: 9 patients (7 aneuploid, 1 normal, 1 no mitoses)
2 patients: +21, 1q21
Later study: 22 patients (21 aneuploid, 1 normal)
8 patients: +7
5 patients: der(18)
4 patients: 1p36, 6q13
3 patients: +X, +12, +20, +21, +22, 1q21 [two patients with t(1;12)(q21;q24)], 2q21
2 patients: +2, +5, +8, +9, +11, +13, +15, +17, −17, +18, −20, 3p21, 3q22, 3q29, 6q21, 6q23, i(6p), 11p15, 12q13
Patients without t(14;18) (21 patients)*
Early study: 17 patients (12 aneuploid, 4 normal, 1 no mitoses)
3 patients: 1q21, 6q22-q24
2 patients: +X, +3, +5, +9, +12, −15, +16, +18, 3p13-p14
Later study: 22 patients (18 aneuploid, 3 normal, 1 no mitoses)
5 patients: 6q21-22
3 patients: +3, −18, 11q22-q23
2 patients: +2, +12, −14, +18, 1p36, 1q21, 1q25, 2p25, 3p13-p14, 3p21-p22, 3p26, 7p15, 13q13-q14, 20q13

Listing of particular chromosome bands refers to breakpoints due to deletions, translocations, inversions, or duplications. Early study: within 1 month of diagnosis; does not include initial normal bone marrows.

* Three patients had t(8;14)(q32;q21) or variant t(6;8;14)(p21;q24;q32).

studies show that successful serial cytogenetic examinations can be performed in a majority of lymphoma patients: 80% of the successful lymph node samples were karyotypically abnormal, and 41 of 43 patients had at least one abnormal sample, with 32 patients exhibiting two or more abnormal samples. The t(14;18) translocation, which is associated with follicular B-cell lymphoma, is the most frequent chromosomal abnormality in NHL. We observed this translocation in 22 of our patients, most of whom (18 of 22) had low-grade disease, either small cleaved cell or mixed small cleaved and large-cell follicular lymphoma. None of these patients were observed to have lost this translocation in subsequent sampling, and all but two of the patients showed additional clonal chromosomal abnormalities, either initially or later in the disease. The presence of t(14;18) was a significant positive prognostic indicator, especially in the low-grade lymphomas (Fig 6).

The most common chromosomal abnormalities observed in our patients, both with and without t(14;18), are shown in Table 3. In studies done early in the disease (within 1 month of diagnosis) in patients with t(14;18), the most frequent additional abnormalities were +21 and involvement of 1q21 (two patients each). In follow-up studies performed later in the disease in the t(14;18) patients, the most frequent abnormality was +7 (eight patients), followed by der(18) (five patients), breakpoints involving 1p36 and 6q13 (four patients each), breakpoints at 1q21 and 2q21 and gain of X, 12, 20, 21, and 22 (three patients each). Most patients acquired additional (secondary) chromosome abnormalities as their diseases progressed. Although no correlation could be made between the type of abnormality and (1) the specific treatment (chemotherapy or radiation) the patient had received or (2) response to treatment, the acquisition of secondary abnormalities was a significant negative prognostic indicator (Fig 6). Secondary chromosomal changes were seen more frequently in patients with t(14;18) (19 of 22 patients) than in patients without this translocation (11 of 21 patients; Fig 3). The additional abnormalities varied considerably from patient to patient, and only a few were seen in more than two patients. All four patients whose lymphoma progressed from indolent to aggressive disease had t(14;18), and all acquired additional clonal abnormalities during the course of their disease. A der(18) was seen in two patients, one of whom also had deletion of 6q, +7, and +12. A third patient had +7, +12, and +18 (among other chromosome gains). All of these abnormalities have been associated with disease progression and/or short survival.[8,21] However, none were of prognostic significance in this group of patients (+7, P_2 = .26; +7 as secondary abnormality, P_2 = .23; +12, P_2 = .49; +7 *v* no +7 in low-grade versus intermediate-/high-grade, P_2 = .97 and .67, respectively).

Twenty-one patients in the present study had chromosomal abnormalities without t(14;18). In early studies (Table 3), breakpoints at 1q21 and 6q22-q24 were seen in three patients each, while +3, −18, 6q21-q22, and 11q22-q23 were most involved (three to five patients each) in the studies performed later in the disease.

Structural abnormalities of chromosome 1 are found in many malignancies, and have been observed in 25% to 50% of NHL patients.[22] In the present series, almost half of the patients had chromosome 1 abnormalities: 10 of 22 patients with t(14;18) and 9 of 21 patients without t(14;18). The most frequently reported breakpoint is 1p36, which has been

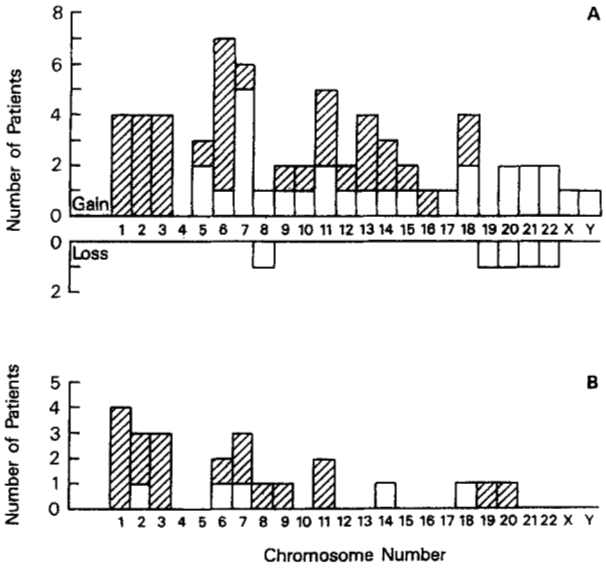

Fig 3. Frequency of secondary chromosomal abnormalities. (A) Patients with t(14;18); (B) patients without t(14;18). (□), whole chromosome gain or loss; (■), structural abnormalities.

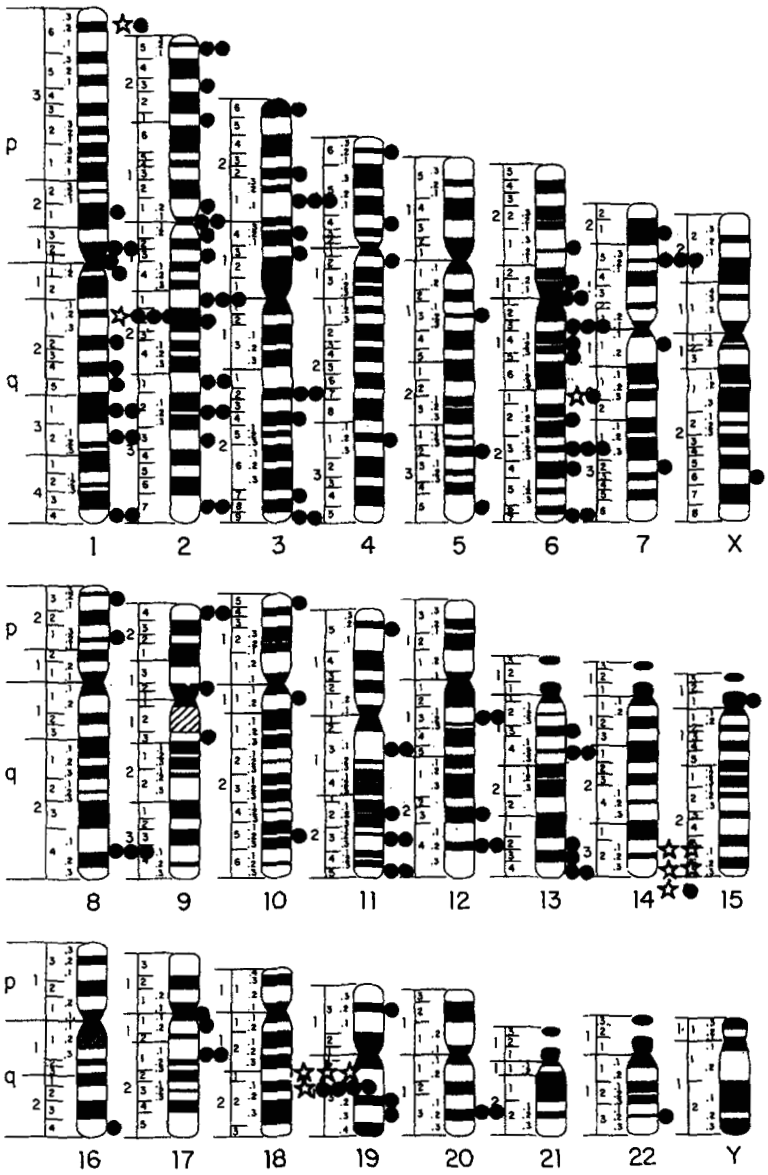

Fig 4. Breakpoint distribution in 43 patients with serial lymph node studies (leukemic breakpoints in case 13 are not included). Each dot represents one patient; each star represents five patients.

associated with t(14;18).[8,18] Six of our patients (cases 5, 10, 12, 14, 25, and 36) had breakpoints involving 1p36: four of them had t(14;18), and in three of these four, it was seen as a secondary change. Histologic type in the six patients ranged from low-grade to high-grade, and only one patient had a short (9 months) survival. Abnormalities of 1q were actually more frequent in this study (15 patients *v* eight with 1p involvement), with the most common breakpoint being 1q21-q22, seen in nine patients. Five of the nine had t(14;18), low- to high-grade lymphoma, and long survivals (greater than 90 months in four of the five cases). One of the five patients showed tumor progression, and two other patients, both of whom had long survivals, had t(1;12)(q21;q24). All four cases of 1q21-q22 without t(14;18) either presented with (two patients) or progressed to (two patients) intermediate- or high-grade histology. Also, all five cases with short survivals had chromosome 1 involvement; however, no two breakpoints were the same. Chromosome 1 aberrations are thought to be secondary in nature and are considered part of clonal evolution,[11] but we observed 1q abnormalities within 1 month of diagnosis in 6 of the 15 patients with 1q involvement. Other reports have indicated their association with poor prognosis and short survival.[8,9] Our studies indicate that 1q involvement is significant only in patients with intermediate-/high-grade lymphoma (P_2 = .0078 versus P_2 = .82 in low-grade lymphoma; Fig 7).

Deletion of 6q is frequently found in lymphomas and in other lymphoid malignancies, such as acute lymphocytic leukemia (ALL) and CLL. In most cases, it is one of several aberrations and is not specific to any particular subtype of NHL.[22] Yunis et al[21] determined that, when accompanied by complete or partial trisomy 7 and/or trisomy 12, deletion

Table 4. Molecular Breakpoint Correlation: t(14;18) and *bcl-2* Rearrangement

Patient No.	Diagnosis	Cell Phenotype	Cytogenetic	MBR	MCR	Survival (mos)
1	Follicular lymphoma	B	t(14;18)	+	–	93
2	Follicular lymphoma	B	t(14;18)	–	–	119+
3	Follicular lymphoma	B	t(14;18)	+	–	128+
4	Follicular lymphoma	B	t(14;18)	–	+	181+
5	Follicular lymphoma	B	t(14;18)	+	–	92+
8	Follicular lymphoma	B	t(14;18)	–	+	69+
11	Follicular lymphoma	B	t(14;18)	+	–	110+
13	Diffuse mixed lymphoma	—	t(14;18)	+	–	64
14	Follicular lymphoma	B	t(14;18)	+	–	64+
15	Large-cell lymphoma	B	t(14;18)	NA	NA	31
16	Follicular lymphoma	B	t(14;18)	+	–	110
22	Follicular lymphoma	B	—	+	–	86+
24	Small noncleaved lymphoma	B	t(14;18)	+	–	180
25	Follicular lymphoma	B	t(14;18)	–	+	30+
26	Follicular lymphoma	B	—	+	–	20
29	Small lymphocytic	B	No 14q+	–	–	72
33	Follicular lymphoma	B	t(3;14;18)	–	+	142+
35	Small noncleaved lymphoma	B	t(14;18)	–	–	12
36	Follicular lymphoma	B	t(14;18)	+	–	158+
37	Follicular lymphoma	B	t(14;18)	+	–	75
39	Follicular lymphoma	B	t(14;18)	–	+	56
41	Follicular lymphoma	B	t(14;18)	–	–	197+
42	Follicular lymphoma	B	t(14;18)	+	–	122+
43	Follicular lymphoma	B	t(14;18)	–	–	77+

Abbreviation: NA, not adequate.

Table 5. Illustrative Survival Results in 43 Cases of Lymphoma

Curve Description	No. of Patients	3-Year Estimate % (95% CI)	6-Year Estimate % (95% CI)	P_2
Low-grade	25	96.0 (80.5; 99.3)	83.6 (64.6; 93.4)	
Int/high	18	38.9 (20.3; 61.4)	27.8 (12.5; 50.9)	<.0001
Low grade; no t(14;18)	7	85.7 (48.7; 97.4)	53.6 (21.3; 83.1)	.021
Low grade; t(14;18)	18	100 (NA)	94.4 (74.2; 99.0)	
Int/high; no t(14;18)	14	35.7 (16.3; 61.2)	14.3 (4.0; 39.9)	.45
Int/high; t(14;18)	4	50.0 (15.0; 85.0)	25.0 (4.6; 69.9)	
Low-grade; no lq	18	94.4 (74.2; 99.0)	83.0 (60.0; 94.0)	.82
Low-grade; lq	7	100 (NA)	85.7 (48.7; 97.4)	
Int/high; no lq	11	54.6 (28.0; 78.7)	27.3 (9.7; 56.6)	.0078
Int/high lq	7	14.3 (2.6; 51.3)	(NA)	
Low-grade; no 6q del	20	100 (NA)	90.0 (69.9; 97.2)	.70
Low-grade; 6q del	5	80.0 (37.6; 96.4)	80.0 (37.6; 96.4)	
Int/high; no 6q del	13	38.5 (17.7; 64.5)	23.1 (8.2; 50.3)	.47
Int/high; 6q del	5	40.0 (11.8; 76.9)	(NA)	
No 6q deletion	33	75.7 (59.0; 87.2)	60.0 (42.9; 75.0)	
6q del; no t(14;18)	5	40.0 (11.8; 76.9)	(NA)	*
6q del; t(14;18)	5	80.0 (37.6; 96.4)	80.0 (37.6; 96.4)	
No +7	34	67.7 (50.8; 80.9)	49.7 (33.7; 65.7)	.26
+7	9	88.9 (56.6; 98.0)	77.8 (45.3; 93.7)	
No secondary abnormality	12	83.3 (55.2; 95.3)	66.7 (39.1; 86.2)	.13
Secondary abnormality	31	67.7 (50.1; 81.4)	51.2 (34.3; 67.8)	

Abbreviations: CI, confidence interval; Int/high, intermediate-/high-grade; NA, not applicable.

* See legend to Fig 8 for P_2 values.

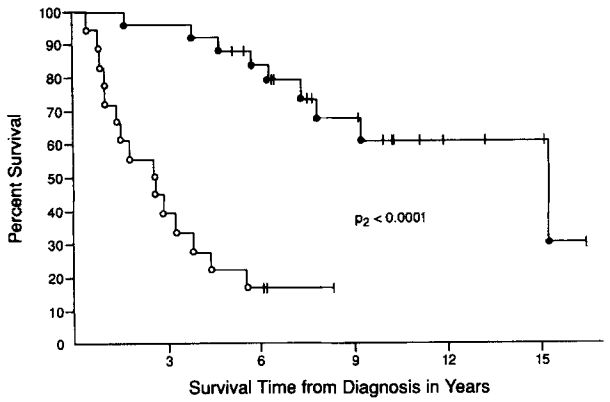

Fig 5. Actuarial survival from time of diagnosis of 43 patients with lymphoma: low-grade (●) versus intermediate-/high-grade (○; 9 of 25 patients died *v* 15 of 18 patients died, respectively).

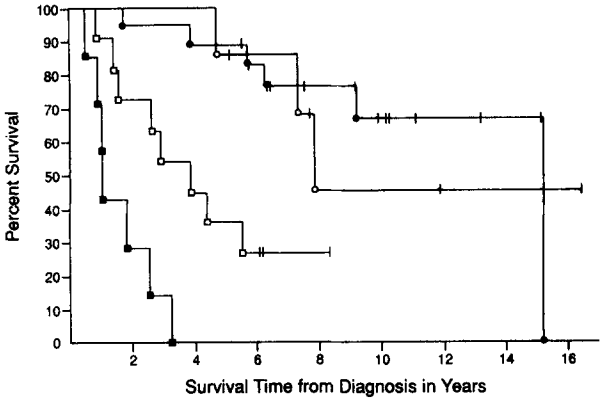

Fig 7. Actuarial survival from time of diagnosis of 43 patients with lymphoma: absence and presence of chromosome 1q involvement in low-grade versus intermediate-/high-grade; in low grade, 1q (○) versus no 1q (●), P_2 = .82; in intermediate-/high-grade, 1q (■) versus no 1q (□), P_2 = .0078.

of 6q was associated with aggressive mixed- or large-cell follicular lymphoma, and Offit et al[8] concluded that breaks at 6q21-q25 predicted a decreased probability of achieving remission. Although some have suggested that 6q deletions are associated with previous treatment[23] or immunoblastic lymphoma,[7] the findings of Hammond et al[10] suggested an association with low-grade disease. A total of 13 patients in our series had involvement of 6q (from 6q13-q27), 10 of whom had 6q deletion. No correlations among lymphoma histology (low-grade to high-grade lymphoma), presence of t(14;18), or survival (median, 46 months; range, 9 to 142+ months) were noted in these patients when taken as a whole. However, when the t(14;18) and non-t(14;18) groups were examined separately, it was observed that patients without t(14;18) were more likely to have a 6q deletion in the initial sample and early in the disease (three of the five patients versus none in the 14;18 group) and that they were more likely to have intermediate- or high-grade histology and shorter survivals than the 6q− patients with t(14;18) (median, 22 months *v* 93 months). When various 6q parameters were examined for prognostic importance, the most significant was the presence of a 6q deletion in patients without the t(14;18) (Table 5 and Fig 8). Five of the 10 patients with 6q deletions had +7 and/or +12; their survivals were not particularly shorter than the other patients with 6q deletions.

Trisomy 7 is one of the most frequent numerical abnormalities in NHL and is most often an additional aberration. In our series, +7 was associated almost exclusively with t(14;18) (eight of nine patients); it was the single most frequent abnormality seen in later disease (eight patients; Table 3) and the most frequent secondary chromosome abnormality (Fig 3). In their study of 434 NHL patients, Offit et al[9] noted that +7 was more frequent in intermediate- to high-grade tumors and less frequently seen in low-grade tumors. In contrast to their findings, eight of our nine patients with +7

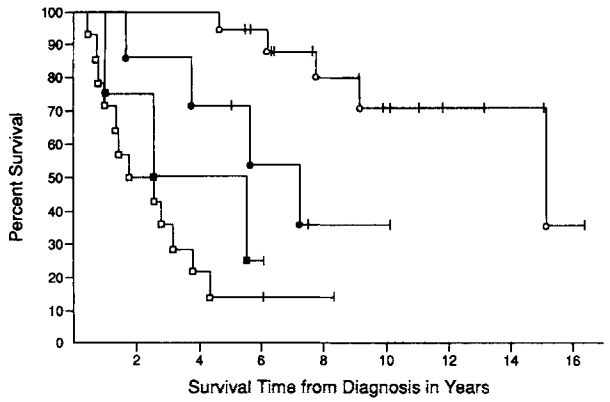

Fig 6. Actuarial survival from time of diagnosis of 43 patients with lymphoma: absence and presence of the translocation t(14;18) in low-grade versus intermediate-/high-grade; in low-grade, t(14;18) (○) versus no t(14;18) (●), P_2 = .021; in intermediate-/high-grade, t(14;18) (■) versus no t(14;18) (□), P_2 = .45.

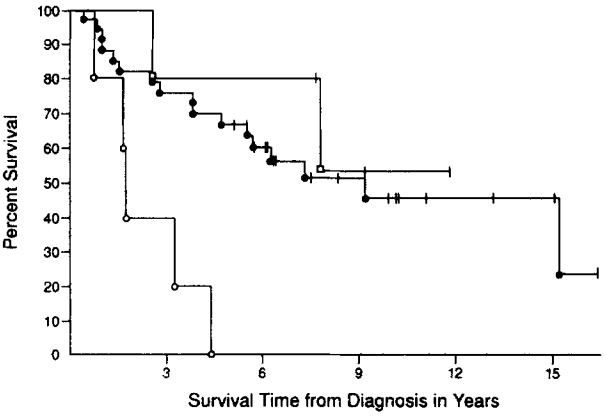

Fig 8. Actuarial survival from time of diagnosis of 43 patients with lymphoma: absence of 6q deletions, ●; presence of 6q deletions with t(14;18), □; presence of 6q deletions without t(14;18), ○. ● versus ○, P_2 = .0014; ● versus □, P_2 = .59; □ versus ○, P_2 = .013.

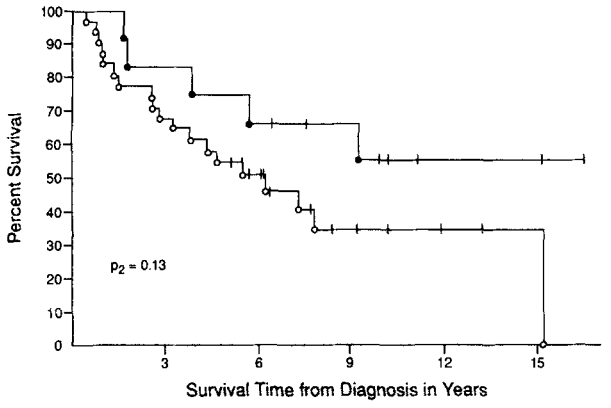

Fig 9. Actuarial survival from time of diagnosis of 43 patients with lymphoma: absence (●) and presence (○) of secondary chromosomal abnormalities (5 of 12 and 19 of 31 patients died, respectively).

originally presented with low-grade tumors; at the time +7 was observed, five of these patients still had low-grade tumors, either follicular small cleaved cell or mixed small cleaved and large-cell. However, two of these five patients showed disease progression to intermediate-grade (follicular, predominantly large-cell). The median survival in these nine patients was 106.2 months (range, 30 to 182 months). Although +7 was observed in the initial study in four patients, in all nine patients the sample was obtained later in the patients' diseases. Statistical analysis of +7 showed little prognostic significance, either as a primary (P_2 = .26) or as a secondary abnormality (P_2 = .23), regardless of grade (low grade, P_2 = .97; intermediate-/high-grade, P_2 = .67). The epidermal growth factor receptor is located on chromosome 7, and, because +7 is also seen in other malignancies such as melanoma and renal cell carcinoma[24] and appears to be associated with late stage disease, it has been suggested that the presence of +7 may confer a selective growth advantage in malignant cells. Levine et al[5] found an association between +7 and breaks in 17q21-25 in follicular large-cell lymphoma. Only two of our patients had 17q breakpoints, neither of whom had +7, t(14;18), or follicular lymphoma.

A der(18), duplication of the 18q marker chromosome derived from the t(14;18) translocation, was seen in five patients in this series; a sixth patient had +18. Yunis et al[21] observed that complete or partial trisomy 18 was associated with large-cell lymphoma. In the current series, however, five of the six patients with additional chromosome 18 material had follicular lymphoma, mixed small cleaved cell and large cell. In three patients it was seen in the initial specimens, two of which were obtained in early disease. In the other two patients, it was a secondary abnormality observed later in disease. Median survival for these six patients was 102 months; however, median survival after first observation of der(18) was 30 months. It appears that this abnormality is associated with disease progression (two of our patients progressed to follicular, predominantly large-cell, and a third patient progressed to high-grade lymphoma); however, the presence of der(18) had no significant effect on overall survival (P_2 = .56).

Other abnormalities of interest seen in several patients each included breaks at 2q21, 3q27-q29, and 11q22-q25. Three patients (cases 1, 5, and 39) had breaks at 2q21. All three had low-grade follicular lymphoma and t(14;18). Survivals ranged from 56 to 93 months, and in all cases, the 2q21 abnormality was seen as a secondary change later in disease. Only Levine et al[5] have noted 2q changes in lymphoma, and in their patients it was associated with diffuse large-cell lymphoma. Abnormalities of 3q27-q29 were noted in three of our patients (cases 1, 15, and 33). All three had t(14;18), all showed 3q involvement later in disease, and in two of the patients, it was a secondary abnormality. Lymphoma type and survival were variable. Previous reports[25-27] have implicated 3q27 involvement in B-cell NHL. Offit et al[25] found t(3;22)(q27;q11) to be associated with diffuse, predominantly large-cell NHL, with no effect on survival, and Bastard et al[26] reported 3q27 in both diffuse, large-cell–type and follicular lymphoma, t(3;14)(q27;q32) being the most common translocation. More recently, Offit et al[27] have studied the clinical outcome of 23 cases of diffuse large-cell lymphoma with *bcl*-6 rearrangements that involved cytogenetic 3q27 translocations in 11 cases. These lymphomas had a propensity to involve extranodal sites and had a better prognosis than large-cell lymphomas without *bcl*-6 rearrangements. None of these cases had t(14;18).

Five of our patients (cases 6, 8, 9, 34, and 40) had breaks in the 11q23-q25 region; one case had Burkitt's lymphoma and a complex translocation with an HSR, t(11;HSR;2). Histologic type, clinical course, and survival were highly variable in the five patients: 11q abnormalities were seen early and later in disease, as both initial and secondary aberrations, and with and without t(14;18). Involvement of 11q has been reported infrequently in lymphoma, with most cases exhibiting breakpoints more proximal to the centromere at either 11q13[28] or 11q21.[29,30]

Figure 4 shows the overall breakpoint distribution in the 43 cases in this series. Chromosomal translocations can be detected with DNA probes in Southern blot analysis without the use of conventional cytogenetics. The *bcl*-2 gene of chromosome 18 is translocated adjacent to one of the J_H genes on chromosome 14 in follicular lymphoma.[31,32] The value of a translocating chromosomal probe is exemplified with the *bcl*-2 gene, whereby DNA hybridization of patient samples serves as an indicator that a t(14;18) translocation has oc-

Table 6. Results of Cox Proportional Hazards Model Analysis

Variable	Parameter Estimate	*P*	RR	95% CI for RR
Low grade				
No t(14;18)	1.53	.035	4.62	1.67, 54.8
Intermediate/high grade				
Age ≤29 yrs	0.74	.012	2.10	1.18, 3.74
1q	2.24	.0026	9.39	2.20, 40.06

Abbreviation: RR, relative risk.

curred. Using a battery of probes (MBR and MCR), we compared the molecular rearrangements with the cytogenetic results in 24 cases; all but two patients in this group had t(14;18) (Table 4). Only a few discrepancies between the molecular and cytogenetic results were noted: two patients had t(14;18) but no molecular rearrangements (either at MBR or MCR), while two patients (cases 22 and 26) had no visible t(14;18) but were positive for MBR rearrangement. The presence of MBR or MCR rearrangements appears to have no effect on survival (P_2 = .60 and P_2 = .57 for MBR and MCR effect, respectively). Whether or not these patients had breaks and rearrangements in areas outside of the regions easily screened by the available probes remains to be determined, but other reasons for the survival benefits, if any, of the presence of rearrangements should be investigated further.

APPENDIX

Cytogenetic Results in Serial Lymph Node Studies in 43 Patients Studied Between 1981 and 1991 by Pathologic Classification According to Initial Diagnosis

Case No.	Survival (mos)*	No. of Specimens†	Diagnosis‡	No. mos Post-diagnosis	Cytogenetic Results (clonal abnormalities)§
Low grade					
12	84	4	A	77	45,XY,t(1;2)(p36;q22),−8,−18/46,XY,t(2;13)(q22;q34)
			H	81	90–92,XXYY,del(1)(q21),i(1q),t(1;3)(p11;p26),t(2;6)(q37;p21),del(3)(p21)
34	46	3	A	1	47–48,XX,+9,+13,+20
			A	14	46,XX/46,XX,del(11)(q22)
21	122+	2	A	51	46,XX
			A	57	46,XX
29	72	5	A	42	No 14q+ (poor)
				42	NM (Sp)
			F	63	NM
			F	70	46,XX,t(2;6)(q31;q27),t(6;9)(q21;q13) (BM)
3	128+	2	B	1	46,XX,+16,−22,t(14;18)(q32;q21)
				48	46,XX
4	181+	3	(initial diagnosis: B)		
			C	76	NM
			D	102	49–51,XXXX,+1,+7,t(7;11)(p15;q13),t(9;11)(p24;q13),t(14;18)(q32;q21),+13,−14,−17,−20
5	92+	4	B	0	48,XY,−6,+12,der(13)t(1;13)(q21;q32),−13,t(14;18)(q32;q21),+17,+19,+21
			B	84	48–50,XY,t(1;2)(p36;q21),+del(6)(q13),+i(6p),+7,−13,t(14;18)(q32;q21),+20,−21
11	110+	4	C	37	47,XXX,t(6;8)(q13;p23),t(14;18)(q32;q21)
			B	83	46,47,XX,t(14;18)(q32;q21),+21
			B	102	47,XX,+del(6)(q13),del(9)(p12)(x2),t(14;18)(q32;q21),+18,−19,−19,+21,−22
16	110	7	B	75	46,XY/46,XY,t(14;18)(q32;q21) (BM)
			B	79	46,XY (BM)
	(leukemic phase)		B	93	NM (PB)
				94	46,XY (PB)
			C	94	NM (LN-frozen)
			C	108	46,XY,t(14;18)(q32;q21) (Sp)
			C	112	46,XY (PB)
31	76+	2	B	0	46,XX/47,XX,+9
			B	42	48,XX,+9,t(14;18)(q32;q21),+der(18)
36	158+	6	B	12	NM
			B	32	46,XX,t(14;18)(q32;q21)
			B	63	46,XX,t(14;18)(q32;q21)
			C	78	NM
			C	98	46,47,XX,t(1;5)(p36;q13),t(5;6)(q13;q23),t(14;18)(q32;q21)
			C	107	46–48,XX,del(5)(q13),t(14;18)(q32;q21)
41	197+	5	B	108	48,XX,+der(12)t(1;12)(q21;q24),t(14;18)(q32;q21)
				115	NM (Sp)
			B	122	46,XX,t(14;18)(q32;q21)
			C	159	46,XX
6	61+	3	B	0	47,XXXX,t(1;6)(q21;q27),−15,+16,i(17q)
			D	36	48–52,XXX,t(1;11)(q21;q25),+2,+7,i(17q)
1	93	4	C	44	49,XY,dup(1)(q22q44),del(6)(q21),+7,+12,t(14;18)(q32;q21),+der(18)
			C	76	46,XY/106,XXYY,t(2;6)(q21;q14),t(2;16)(p11;q24),del(6)(q14),del(6)(q23),t(14;18)(q32;q21)
			D	90	46,XY,t(2;16)(p11;q24),t(3;7)(q29;q11),t(14;18)(q32;q21)/104,XXY,t(2;6)(q21;q14),t(3;7)(q29;q11),i(6p),del(6)(q14),del(6)(q23),t(14;18)(q32;q21) (Sp)
2	119+	4	C	36	46,XY,t(14;18)(q32;q21)
			C	44	46,XY
			C	65	46,XY,t(14;18)(q32;q21)
14	64+	3	C	0	NM
			C	25	46,XY,dup(12)(q13q23),t(14;18)(q32;q23)
			D	54	46,XY,t(1;3)(p36;p21),dup(12)(q13q23),t(14;18)(q32;q21)

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APPENDIX

Cytogenetic Results in Serial Lymph Node Studies in 43 Patients Studied Between 1981 and 1991 by Pathologic Classification According to Initial Diagnosis (Cont'd)

Case No.	Survival (mos)*	No. of Specimens†	Diagnosis‡	No. mos Post-diagnosis	Cytogenetic Results (clonal abnormalities)§
24	180	2	(initial diagnosis: C)		
			J	173	50,XY,del(3)(q22),+7,t(14;18)(q32;q21),t(17;19)(q11;q13.3) (AF)
			J	180	50,XY,t(3;13)(q22;q34),+7,+8,t(14;18)(q32;q21),t(17;19)(q11;q13.3),+der(18),−20,+21
25	58+	2	C	0	46,XXYY,t(1;2)(p36;p21),i(2q),−8,t(14;18)(q32;q21)
			C	52	50–54, 77–78,XXY,t(1;2)(p36;p21),+5,t(6;15)(p11;p11),+7,+11,t(14;18)(q32;q21),+22
33	142+	6	C	50	47(no furth analy poss)
			C	60	NM
			C	95	47,XX,t(3;14)(14;18)(q29;q13q32;q21),der(12)t(1;12)(q21;q24),del(6)(q21)
			C	127	46,48,XX,t(3;14)(14;18)(q29;q13q32;q21),der(12)t(1;12)(q21;q24),del(6)(q21),+15
37	75	4	C	45	46,47,XX,t(14;18)(q32;q21),+der(18)
			C	71	47–48,XX,t(14;18)(q32;q21),+17,+der(18)
39	56	2	C	24	46–50,XY,+2,+7,+9,+12,t(14;18)(q32;q21),+15,+17,+21,+22
			D	54	46,XY/48–54,XXY,del(1)(q32),t(1;2)(q24;q21),+10,+11,+12,+13,+13,t(14;18)(q32;q21),+18,+20
42	122+	4	C	53	48,49,XX,+2,+8,+20,t(14;18)(q32;q21)
			C	69	49,XX,+2,+12,t(14;18)(q32;q21),+22
			C	111	46,50–52,XX,+2,+7,+8,+14,t(14;18)(q32;q21),+20
43	77+	6	C	0	47,XY,t(14;18)(q32;q21),+der(18)
			C	28	46,XY/47,XY,t(14;18)(q32;q21),+der(18)
			C	38	47,XY,t(14;18)(q32;q21),+der(18)
			C	39	46,XY/47,XY,t(14;18)(q32;q21),+der(18) (BM)
26	20	3	C	7	46,83–90,XY,del(6)(q23)
			No tumor seen	15	NM
22	86+	4	C	26	46,XX,r
			C	34	46,XX
			E	55	46,XX
Intermediate grade					
8	69+	2	D	0	46,XY,inv(9)(p12q13)
			D	12	46,47,XYY,inv(9)(p12q13),t(11;12)(q25;q13),t(14;18)(q32;q21)
32 AILD-like T-cell lymphoma	18	3	F	2	46,XY
				6	46,XY,del(8)(p21)
			F	14	46,47,XY,del(8)(p21)
13	64	4	G	57	84–88,XY,t(14;18)(q32;q21)
			G	60	NM
			2° leukemia	64	43–44,46,XY,del(5)(q13q33),del(7)(q21.3),−18,−21 (PB)
19	39	2	G	0	48,49,X,t(X;19)(q26;q13),t(1;9)(9qter→9pter::1p11→1qter::1q21→1qter),−1,+2,+3,+3,t(3;4)(p14;p16),del(6)(q22),−14
			J	10	47,50,XX,t(1;9)(9qter→9pter::1p11→1qter::1q21→1qter),+3,+3,t(3;4)(p14;p16),+6,del(6)(q22),−14,+18
10	9	2	G	0	45,mar
			G	7	44,46,XY,del(1)(p36),del(2)(q32),del(5)(q32),+del(6)(q15q21),t(8;14)(q24;q32),+12,−14
7	12	2	G	0	46,XX (PF)
			G	11	43,X,−X,del(1)(q25),del(3)(p22)x2,del(13)(q13),−17,−18
18	22	2	G	1	47,XXY,der(1)t(1;13)(q32;q14),del(6)(q21),der(22)t(1;22)(q21;q13)
			J	6	46,47,XXY,der(1)t(1;13)(q32;q14),del(6)(q21),der(22)t(1;22)(q21;q13)
27	48	3	G	16	50–51,XY,+1,t(2;4)(p25;q12),−7,+11,+12,−13,+15,t(17;19)(q21;p13)
			D	29	NM
15	31	4	G	0	46,47,XY,del(1)(p21),del(3)(q22),t(14;18;1)(q32;q21;q31)
			D	14	46,XY
			G	26	50,XY,del(1)(p21),+t(2;3)(q31;q27);ider(2)t(2;3)(q37;p21),+5,+6,del(6)(q13),+7,−8,del(10)(q25),t(14;18;1)(q32;q21;q31),+r
28	31	6	WA	1	48/X,+X,r(Y),t(6;8;14)(p21;q24;q32),del(20)(q13)
			ML, NOS	21	46,X,−Y,t(3;3)(p26;q21),t(6;8;14)(p21;q24;q32),del(20)(q13)
			ML, NOS	30	46,68–84,t(6;8;14)(p21;q24;q32),del(20)(q13)
High grade					
9	34	3	H	15	46,XX/50,XX,+3,t(3;10)(p13;p15),del(4)(q31),+del(14)(q24),+16,+18
			H	26	48,XX,+3,t(3;10)(p13;p15),del(4)(q31),del(11)(q13q23),del(14)(q24),+18 (Sp)

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APPENDIX

Cytogenetic Results in Serial Lymph Node Studies in 43 Patients Studied Between 1981 and 1991 by Pathologic Classification According to Initial Diagnosis (Cont'd)

Case No.	Survival (mos)*	No. of Specimens†	Diagnosis‡	No. mos Post-diagnosis	Cytogenetic Results (clonal abnormalities)§
30 Ki-1 lymphoma	10	9	H	0	NM
			H	0	45,46,XY,inv(2)(p25q13),der(2)t(2;10)(p23q11;q11),del(4)(p14),der(5)t(2;5)(p23;q35),del (20)(q13),−21 (PF)
			H	1	NM
			H	1	46,47(same markers as previously) (PF)
			H	5	NMx2 (CSF)
			H	9	NG (PF)
			H	10	42,43,XY,inv(2)(p25q13),der(5)t(2;5)(p23;q35),der(9)t(1;9)(q11;p24),der(20)t(14;20) (q11;q13)(brain lesion)
17 Precursor T-cell lymphoblastic lymphoma	16	3	I	0	45,XY,−15
			I	8	46,XY
			No tumor seen	11	46,XY,+2,−15 (Sp)
23 Precursor T-cell lymphoblastic lymphoma	69+	5	I	0	46,XY
			I	10	46,XY
			I	13	46,XY/46,XY,inv(7)(p21p15)
			I	19	46,XY
38 Precursor T-cell lymphoblastic lymphoma	50	5	I	0	49,XY,+5,del(6)(q24),+9,+12,14q+
			I	19	NM (PF)
			I	40	49,XY,t(3;7)(q24;p15)
			I	41	NM (PF)
			I	50	46,XY,t(3;7)(q24;p15),t(3;8)(q24;q24) (PB)
35	12	5	J	0	46,47,XY,del(1)(q21),+del(1)(q31),t(14;18)(q32;q21),−17,+21
				0	NM (PF)
			H	11	45,46,XY,t(11;13)(p15;q14),t(14;18)(q32;q21),−17
			No tumor seen	11	NM (Sp)
40	5	3	J	0	45,46,XX,−9,t(11;HSR;2)(q23;HSR;q33),t(8;14)(q24;q32) (BM)
			J	5	NM
			J	5	46,XX,der(7)t(2;7)(q32;q32),t(8;14)(q24;q32),der(11)t(11;HSR;2)(q23;HSR;q33), der(19)t(1;19)(q25;q13) (PB)
20	100+	2	J	0	46,XY
		Lymphoid hyperplasia		33	47,XY,+3

All patients had B-cell lymphomas except as noted in the first column.

Abbreviations: BM, bone marrow; PB, peripheral blood; Sp, spleen; PF, pleural effusion; AF, ascites fluid; CSF, cerebrospinal fluid; NM, no mitoses or no analyzable mitoses; WA, Wiscott-Aldrich syndrome; ML, NOS, malignant lymphoma with plasmacytoid features, not further subclassified.

*Survival from initial diagnosis.

† Total number of specimens, including normal bone marrows and peripheral blood not shown under Cytogenetic Results.

‡ See Table 2 for letter code definitions of pathologic diagnosis.

§ All specimens were lymph nodes except as noted.

REFERENCES

1. Dohner H, Bloomfield CD, Frizzera G, Frestedt J, Arthur DC: Recurring chromosome abnormalities in Hodgkin's disease. Genes Chromosom Cancer 5:392, 1992

2. Whang-Peng J, Lee EC: Cytogenetics, Magrath I (ed): Non-Hodgkin's Lymphomas. London, UK, Edward Arnold, 1990, p 77

3. Armitage JO, Sanger WG, Weisenburger DD, Harrington DS, Linder J, Bierman PJ, Vose JM, Purtilo DT: Correlation of secondary cytogenetic abnormalities with histologic appearance in non-Hodgkin's lymphomas bearing t(14;18)(q32;q21). J Natl Cancer Inst 80:576, 1988

4. Juneja S, Lukeis R, Tan L, Cooper I, Szelag G, Parkin JD, Ironside P, Garson OM: Cytogenetic analysis of 147 cases of non-Hodgkin's lymphoma: Non-random chromosomal abnormalities and histological correlations. Br J Haematol 76:231, 1990

5. Levine EG, Juneja S, Arthur D, Garson OM, Machnicki JL, Frizzera G, Ironside P, Cooper I, Hurd DD, Peterson BA, Mosel D, Bloomfield CD: Sequentional karyotypes in non-Hodgkin's lymphoma: Their nature and significance. Genes Chromosom Cancer 1:270, 1990

6. Levine EG, Bloomfield CD: Cytogenetics of non-Hodgkin's lymphoma. J Natl Cancer Inst Monogr 10:7, 1990

7. Schouten HC, Sanger WG, Weisenburger DD, Anderson J, Armitage JO: Chromosomal abnormalities in untreated patients with non-Hodgkin's lymphoma: Associations with histology, clinical characteristics, and treatment outcome. Blood 75:1841, 1990

8. Offit K, Wong G, Filippa DA, Tao Y, Chaganti RSK: Cytogenetic analysis of 434 consecutively ascertained specimens of non-Hodgkin's lymphoma: Clinical correlations. Blood 77:1508, 1991

9. Offit K, Jhanwar SC, Ladanyi M, Filippa DA, Chaganti RSK: Cytogenetic analysis of 434 consecutively ascertained specimens of non-Hodgkin's lymphoma: Correlations between recurrent aberrations, histology, and exposure to cytotoxic treatment. Genes Chromosom Cancer 3:189, 1991

10. Hammond DW, Goepel JR, Aitken M, Hancock BW, Potter AM, Goyns MH: Cytogenetic analysis of a United Kingdom series of non-Hodgkin's lymphomas. Cancer Genet Cytogenet 61:31, 1992

11. Fifth International Workshop on Chromosomes in Leukemia-Lymphoma: Correlation of chromosome abnormalities with histologic and immunologic characteristics in non-Hodgkin's lymphoma and adult T-cell leukemia-lymphoma. Blood 70:1554, 1987

12. The Non-Hodgkin's Lymphoma Pathologic Classification Project: National Cancer Institute sponsored study of classifications of non-Hodgkin's lymphomas. Summary and description of a working formation for clinical usage. Cancer 49:2112, 1982

13. ISCN (1991), Mitelman F (ed): Guidelines for Cancer Cytogenetics, Supplement to An International System for Human Cytogenetic Nomenclature. Basel, Switzerland, Karger, 1991

14. Yano T, van Krieken JHJM, Magrath IT, Longo DL, Jaffe ES, Raffeld M: Histogenetic correlations between subcategories of small noncleaved cell lymphomas. Blood 79:1282, 1992

15. Kaplan E, Meier P: Non-parametric estimation from incomplete observations. J Am Stat Assoc 53:457, 1958

16. Mantel N: Evaluation of survival data and two new rank order statistics arising in its consideration. Cancer Chemother Rep 50:163, 1966

17. Cox D: Regression models and lifetables. J Stat Soc (B) 34:187, 1972

18. Matthews DE, Farewell VT: Using and Understanding Medical Statistics. Basel, Switzerland, Karger, 1985, p 148

19. Fischer P, Nacheva E, Mason DY, Sherrington PD, Holye C, Hayhoe FGJ, Karpas A. A Ki-1 (CD30)-positive human cell line (Karpas 299) established from a high-grade non-Hodgkin's lymphoma, showing a 2;5 translocation and rearrangement of the T-cell receptor B-chain gene. Blood 72:234, 1988

20. Ebrahim SAD, Ladanyi M, Desai SB, Offit K, Jhanwar SC, Filippa DA, Lieberman PH, Chaganti RSK: Immunohistochemical, molecular, and cytogenetic analysis of a consecutive series of 20 peripheral T-cell lymphomas of uncertain lineage, including 12 Ki-1 positive lymphomas. Genes Chromosom Cancer 2:27, 1990

21. Yunis JJ, Frizzera G, Oken MM, McKenna J, Theologides A, Arnesen M: Multiple recurrent genomic defects in follicular lymphoma. A possible model for cancer. N Engl J Med 316:79, 1987

22. Heim S, Mitelman F: Cancer Cytogenetics. New York, NY, Alan R. Liss, 1987

23. Cabanillas F, Pathak S, Manning J, Katz R, McLaughlin P, Velasquez WS, Hagemeister FB, Goodacre A, Cork A, Butler JJ, Freireich EF: Frequent non random chromosome abnormalities in 27 patients with untreated large cell lymphoma and immunoblastic lymphoma. Cancer Res 48:5557, 1988

24. Sandberg AA: The Chromosomes in Human Cancer and Leukemia (ed. 2). New York, NY, Elsevier, 1990

25. Offit K, Jhanwar S, Ebrahim SAD, Filippa D, Clarkson BD, Chaganti RSK: t(3;22)(q27;q11): A novel translocation associated with diffuse non-Hodgkin's lymphoma. Blood 74:1876, 1989

26. Bastard C, Tilly H, Lenormand B, Bigorgne C, Boulet D, Kunlin A, Monconduit M, Peguet H: Translocations involving band 3q27 and IG gene regions in non-Hodgkin's lymphoma. Blood 79:2527, 1992

27. Offit K, Lo Coco F, Louie DC, Parsa NZ, Leung D, Portlock C, Ye BH, Lista F, Filippa DA, Rosenbaum A, Ladanyi M, Jhanwar S, Dalla-Favera R, Chaganti RSK: Rearrangement of the bcl-6 gene as a prognostic marker in diffuse large-cell lymphoma. N Engl J Med 331:74, 1994

28. Tsujimoto Y, Jaffe E, Cossman J, Gorman J, Nowell PC, Croce CM: Clustering of break point on chromosome 11 in human B cell neoplasms with the t(11;14) chromosome translocation. Nature 315:340, 1985

29. Levine EG, Arthur KJ, Machnicki J, Frizzera G, Hurd D, Peterson B, Gajl-Peczalska HJ, Bloomfield CD: Four new recurring translocations in non-Hodgkin's lymphoma. Blood 74:1796, 1989

30. Griffin CA, Zehnbauer BA, Beschorner WE, Ambinder R, Mann R: t(11;18)(q21;q21) is a recurrent chromosome abnormality in small lymphocytic lymphoma. Genes Chromosom Cancer 4:153, 1992

31. Erikson J, Finan J, Tsujimoto Y, Nowell PC, Croce CM: The chromosome 14 breakpoint in neoplastic B-cells with the t(11;14) translocation involves the immunoglobulin heavy chain locus. Proc Natl Aad Sci USA 81:4144, 1984

32. Tsujimoto Y, Cossman J, Jaffe E, Croce CM: Involvement of the bcl-2 gene in human follicular lymphoma. Science 228:1097, 1985